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Enzymatic Conversion of Biomass for Fuels Production

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Chapter 19

Pectin-Rich Residues Generated by Processing of Citrus Fruits, Apples, and Sugar Beets

Enzymatic Hydrolysis and Biological Conversion to Value-Added Products

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Processing of citrus, apple and beet crops to juice and crystalline sugar annually generates several million tons of residues which are sold as a cattle feed or cause disposal problems. These residues are very rich in carbohydrates and are attractive potential feedstock for microbial conversions to value added liquid fuels and other products. The residues are rich in pectin and in the case of apple pomace and citrus processing residues they also contain large amounts of soluble sugars. All polysaccharides in these residues are easily hydrolysed to monomeric sugars by mixtures of cellulolytic and pectinolytic enzymes. Microbial conversions of sugar rich hydrolysates from these residues will require identification and development of microorganisms that can utilize galacturonic acid and five carbon sugars.

It has been estimated that the annual production of cellulosic biomass could supply 10 times our energy needs and 100 times our food needs on a global scale (*1*). The term cellulosic biomass is usually applied to woody and other lignified tissues of plants which are currently under utilized and undervalued. While there is no doubt that woody residues and residues from harvesting of grain represent the largest reservoir of the cellulosic biomass necessary to supply huge fuel markets (*2,3*), these tissues are heavily lignified and present serious obstacles to efficient enzymatic hydrolysis of cell wall polysaccharides (*3-7*). Lignin hemicellulose complexes shield the cellulose fibers from enzymes and prevent significant hydrolysis of polysaccharides in these walls. Very harsh mechanical or chemomechanical pretreatments of these tissues are necessary for efficient enzymatic hydrolysis to occur, and even then, the crystalline structure of the major polymer, cellulose, creates difficulties in terms of yield, enzyme loading and rates of enzymatic depolymerization (*4,6-9*).

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These serious difficulties with enzymatic hydrolysis of lignified plant cell walls may create an erroneous impression that all plant tissues are very resistant to enzymatic hydrolysis and will require chemical pretreatments.

We would like to present in this short review three examples of specialized cellulosic tissues in fruits and tubers in which lignin is apparently absent, cells are cemented by pectin and the tissues are relatively easy to hydrolyze by mixtures of pectinolytic, hemicellulolytic and cellulolytic enzymes. The three examples are processing residues from the production of citrus, apple and sugar beet juices. These residues have been chosen because they are produced in relatively large amounts by mature industries in the U.S. and other countries. All three residues are available at the processing plants, which potentially decreases collection and transportation problems. Moreover, the carbohydrate composition of cell walls of these residues strongly resembles the composition of cell walls of many other fruits, vegetables and tubers used for human consumption (*10-16*), therefore the issues discussed for these selected residues may have broader applications.

Summary of Processing

Since all three crops are grown for processing to sweet juice, the key processing step is the expression of the juice.

Citrus Juice Products. In the case of citrus fruit two types of extractors are used (*17-22*) which either core a fruit or ream fruit halves and express the juice and pulp with minimal contamination by peel juice and peel oil. Peel juice is astringent and unpalatable and citrus peel oil decreases quality and storability of the juice. Other pressing equipment for whole fruit has been tried (*17,20*) and abandoned because of peel oil contamination problems. The juice is then screened to remove the coarse pulp using finishers (*17,19,21,22*) and either pasteurized by heat treatment or concentrated in multiple effect evaporators before cold storage and packaging. The heat treatment of the juice is extremely important because it not only kills spoilage microorganisms but also inactivates enzymes in the juice which destabilize the particulate cloud. One of these enzymes, pectinmethylesterase, has been implicated in the modification of the citrus juice cloud which leads to its rapid coagulation and other problems. Since cloudiness is one of the very important attributes customers expect in citrus juice products, its maintenance is of great importance to the industry.

The other half of the plant is devoted to the processing of residues (*17,18*). Residues, mainly peel, cores, segment membranes and small amounts of seed amount to approximately one half of the weight of fruit (*17,18*) and due to high sugar content are prone to rapid spoilage. These residues are hammermilled to coarse particles and blended with small amounts of calcium oxide. Calcium oxide reacts with pectin in the cell walls and increases the yield of peel juice. The limed, ground residues are partially dewatered in a screw press. The peel juice is concentrated in multiple effect evaporators using waste heat from the peel dryer. Resulting peel molasses are sometimes sold, but often are blended back with dewatered residue and dried in the peel dryer to a cattle feed. Small amounts of a valuable terpene, limonene, are recovered from the vapors of the evaporator. The pressing of the peel juice and

evaporation to molasses are driven more by the economy of heat utilization and recovery of limonene than by the value of molasses (*23*).

The citrus juice production is dominated by the processing of several varieties of oranges (*24*) with a much smaller amount contributed by grapefruit. One unique feature of orange crop and orange juice production is a very long harvesting season which in Florida lasts approximately from October until May of the next year. The long harvesting season assures an equally long processing season and supply of residues.

Apple Juice Products. Apple fruit processing does not pose a peel oil contamination problem so important to the citrus processing industry. The whole fruit can be disintegrated and pressed by a variety of pressing machines to produce juice and pressed residue called pomace (*25-28*). Diffusion extractors, adapted from the sugar beet industry, are also used (*25*). The juice yield in the pressing step usually decreases with storage of apples, so the apple tissue is often treated with pectinolytic enzymes before the pressing step to help to disintegrate it. One recent trend (*25,28*) involves liquefaction of apple tissues with a mixture of pectinolytic, cellulolytic and hemicellulolytic enzymes which dramatically increases the juice yield. The authenticity of such a product contaminated with sugars from apple cell walls is of course highly questionable (*28*). Since consumers usually expect a clear apple juice product much of the processing is devoted to clarification of the juice using filtration, precipitation (fining) and pectinolytic enzyme treatments. The clarified juice is either pasteurized or evaporated before cold storage and packaging (*25*). The important enzymes that need to be inactivated or inhibited in apple juice products are polyphenol oxidases (*25,28*) which cause darkening of the juice and formation of precipitates during storage. The processing season can vary from 3-4 months to almost a year depending on local conditions. Introduction of extended storage methods for apples extended the length of supply and the processing season to a whole year (*25*). The disposal of the apple pomace depends on local conditions (*25*). It is often used as a supplemental cattle feed or soil conditioner. A few plants use apple pomace for the production of pectin.

Sugar Beet Processing. Production of juice from sugar beets uses a leaching process, rather than the pressing used with fruit juices (*29,30*). Washed beets are sliced into thin pieces called cosettes, which are then leached with hot water, usually in continuous countercurrent diffusers. The beet tissue is heated above 70°C to modify the cell walls and increase their permeability to sucrose (*29,30*). The leached juice is then clarified by liming, carbonation with carbon dioxide, filtration and treatment with sulfur dioxide. The thin, clarified juice is then converted to crystalline sugar and molasses by an additional sequence of processing steps (*29,30*) which include concentration by evaporation, deionization, additional decolorization, and crystallization or precipitation of sucrose with lime. The leached beet cosettes are blended with spent molasses, dried and sold as a cattle feed supplement in the United States (*30*), but in other areas traditional ensiling for cattle feed or spreading on soil may still be used (*31-33*). Drying is the method choice if long term storage and long distance transportation of all three residues is contemplated. The high water content of wet residues makes long distance transportation unattractive.

Availability and Composition of Residues

Citrus juice processing residues are available in the U.S. mainly in the state of Florida. The smaller California-Arizona crop is usually sold on the fresh fruit market. Approximately 600,000 to one million dry tons of citrus processing residues was produced annually in the 1980's (*34*). Additional large amounts of citrus processing residues are available in other major processing countries such as Brazil (a world leader), Mexico, Spain, China and Israel.

Apples are grown in 35 states of the U.S. (*25*) with Washington state by far the largest producer, followed by New York, Michigan, California and Pennsylvania. More than half of the annual crop is sold as fresh fruit and approximately 44% is processed to juice, canned sauce and other products. The processing of apples in the U.S. generates approximately 1.3 million tons of wet, (i.e., approximately 250-400,000 dry) pomace a year (*35*). Many other countries in the world produce and process apples (*25,28*) so additional amounts of pomace are produced in temperate zones.

The annual production of sugar beets in the United States varied between 20 and 30 million wet tons during the last two decades (*36*) from which approximately 1.6-2.5 million dry tons of residue (marc) have been produced. The northern tier of states from Ohio to the Pacific Northwest are the leading producers in the U.S. Even larger quantities of sugar beets are grown and processed in northern Europe and the European part of the former Soviet Union (*29*).

The production statistics indicate that several million tons of these residues accumulate annually on the worldwide basis. These amounts are nowhere near the large quantities of lignocellulosic residues obtainable from the production of grain crops (2) but are sizable enough to be a resource for the chemical industry.

All three processing residues are characterized by a high content of water (Table I). The two fruit processing residues are also characterized by a high content of soluble sugars (Table I). The soluble sugars in apple juice and pomace are fructose, glucose and sucrose with fructose being the dominant component (*25*). Sorbitol is also present in small amounts. Citrus processing residues also contain glucose, fructose and sucrose, but fructose and glucose are present in nearly equimolar amounts (*17*) since they are produced by hydrolysis of sucrose transported by the sap to the fruit. Immature apples contain small amounts of starch (*25*) which disappears with maturity of the fruit. No starch has been detected in citrus fruit and sugar beets. Sucrose, the dominant sugar in sugar beets, is leached during processing so only traces remain in the residue. There is a significant variation in the soluble sugar content of both apple and citrus processing residues, because soluble sugar content increases during maturation of the fruit on the tree and the cell wall material decreases at the same time (*17,25,41*). Other soluble components in these tissues are protein, minerals and organic acids, namely citric in citrus, malic in apples and oxalic in sugar beets.

Despite the crude nature of analytical methods used to obtain results in Table I, the sum of reducing sugar content and alcohol-insoluble solids indicates that all three processing residues are very rich in carbohydrates. The low crude fiber content also indicates that the processing residues have a low content of cellulose and lignin. These conclusions are supported by detailed analyses of solid residues insoluble in aqueous alcohol (usually 80% v/v) or water and aqueous alcohol (Table II). These

Table I.
Approximate Composition of Processing Residues[1]

Component	Residue Weight Percent		
	Apple Pomace[2]	Citrus Peel[2]	Sugar Beet Marc[2]
Water	66-78	70-82	90-93
Total solids	22-34	18-30	7-10[3]
Soluble sugar	6.6-12.2 (30-36)	5.4-12.0 (30-40)	0.2-0.3 (3-4)
Alcohol Insoluble Solids (AIS)	11.0-20.4 (50-60)	8.1-21.0 (45-70)	6.3-9 (90)
Crude fiber	3.3-8.8 (15-26)	2.2-3.6 (12)	1.4-2.0 (20)
Pectin	5.1-7.8 (23)	3.2-6.9 (18-23)	1.4-2.0 (20)
Crude protein	1.0-1.5 (4.4)	1.2-2.2 (6.6-7.3)	0.6-0.9 (8.7)
Fat (ether extract)	1.5-2.3 (6.8)	0.7-1.5 (4-5)	<0.1 (<1)
Ash	0.3-0.5 (1.4)	0.4-1.5 (2.2-5.0)	0.2-0.4 (3-4)

[1]The results are tabulated in weight percent of wet material. The numbers in parenthesis are tabulated in weight percent of dry total solids.
[2]The results for apple pomace; citrus peel; and sugar beet marc are adapted from references *35, 37-40; 18,41-44; 11,16,32,33,45,46*; respectively.
[3]Total solids content can be increased to 15-22% by pressing (*31,32*).

Table II. Composition of Cell Wall Fraction[1,2]

Component[2]	Residue Weight Percent		
	Apple Pomace	Orange Peel	Sugar Beet Marc
Cellulose	20.9	17.5-21.4	22-24
Non-cellulosic glucose	4.3[3]	N.D.[4]	1.8-2.5
Total glucose	25.2-33.3	23.7	21.6-26.5
Galactose	3.0-7.0	8.2	4.2-4.9
Mannose	1.0-3.4	-	0.3-1.5
Arabinose	5.1-14.3	14.2	16.3-20.1
Xylose	5.8-6.6	<5	1.4-1.6
Fucose	0.6-1.2	N.D.	N.D.
Rhamnose	0.3-1.5	<2	1.0-2.25
Total neutral sugars	41.0-67.3	46.1-53.1	44.8-56.8
Galacturonic acid	18.7-28.2	26.0	18.4-23.0
Total sugars	59.7-95.5	72.1-79.1	63.7-79.8
Lignin	N.D.	3.0	1.0-2.0
Protein	9-11	5.8-6.7	3.6-8.0
Ash	1.5-2.0	3.9-4.1	4.4-12.0

[1]Cell wall fraction refers to solid residue insoluble in aqueous alcohol or water and aqueous alcohol. The results for apple pomace; orange peel; and sugar beet marc are adapted from references *10,25,47-49; 44,49; 11,16,45,51;* respectively.
[2]Content of individual sugars is expressed on polymeric (anhydrous) basis.
[3]Non-cellulosic anhydroglucose corresponds to starch content in apple pomace (*25*).
[4]N.D. = not determined.

residues, usually called alcohol-insoluble solids or residues (AIS or AIR) are highly enriched in cell wall components, but are contaminated with small amounts of protein and minerals (Table II).

The cell walls and connecting middle lamella in these tissues are composed of carbohydrates. Lignin, so prevalent in many other plant tissues (e.g. wood) appears to be absent. Pectin, a polymer of galacturonic acid methyl ester is the major component of the middle lamella (*52*) but is also present in cell walls of fruits and tubers. The content or composition of the other polysaccharides are also markedly different from lignified plant tissues. The cellulose content (17-24%) is very low when compared to wood or mature grass tissues (*4*) in which it is approximately 40-50% of the dry weight. The xylan content is also very low. The major hemicelluloses contain arabinose and galactose as their monomeric units. Branched 1,5-α-L-arabinan has been isolated from sugar beet cell walls (*53*) and ß-1,4-D-galactan has been isolated from citrus pectin (*54*), but generally very little is known about composition and structure of major polysaccharides in cell walls of these tissues. This lack of knowledge will complicate the development of efficient systems for enzymatic hydrolysis as discussed below. Discussion of the structure of primary cell walls of flowering plants (*55*) may be applicable to specialized tissues discussed here, but in the absence of solid structural analyses the extrapolation (*56*) from one tissue to another may be misleading. Even the structure of pectins which has been studied for decades has not been unambiguously determined (*57-59*). Despite the lack of detailed knowledge about the structure of cell walls in fruits and tuberous roots, their susceptibility to microbial decomposition and high digestibility in the rumen of cattle have implied for a long time that these tissues are not very resistant to enzymatic hydrolysis. However, the more detailed studies of enzymatic hydrolysis summarized below were not performed until relatively recent times.

Enzymatic Hydrolysis

The first set of polymers that need to be hydrolyzed in fruit and tuber tissues are pectins. Pectins are polymers of α-1,4 linked galacturonic acid and appear to be composed of linear segments of polygalacturonic acid and "hairy" regions containing rhamnose units and side chains of neutral polysaccharides containing arabinose and galactose (*57,58,60-63*). Part of the pectic substances is soluble in water or aqueous solutions of chelating agents. The other part, termed protopectin (*64*) is crosslinked with other polymers and remains insoluble in water. Carboxylic groups in pectic substances are highly esterified with methoxyl groups and pectins from sugar beets, citrus, apple and some other fruit are partially acetylated (*59,65,66*) probably at the C-2 and/or C-3 positions of anhydrogalacturonyl units. Small amounts of ferulic acid esters have also been detected in sugar beet pectins (*11,59,67*).

The hydrolysis of pectic substances requires interaction of several enzymes (*68-70*). Enzymatic cleavage of the polygalacturonic acid backbone is unique among polysaccharides, because it can proceed by two different mechanisms, hydrolysis or ß-elimination. The enzymes that cleave the glycosidic bond of polygalacturonic acid by ß-(trans) elimination are termed polymethylgalacturonate lyases when they cleave esterified (methoxylated) pectin chains or polygalacturonate lyases when they cleave de-esterified polygalacturonic acid (*68*). The combined action of endo- and exo-

polygalacturonate lyases produces short oligomers of galacturonic acid terminated on the non-reducing end by δ-4,5-D-galacturonate residues. The combined action of endo- and exo-polymethylgalacturonate lyases produces similar methoxylated oligomers. Monomeric products can be obtained by action of oligogalacturonate lyases (*68,71*). All lyases have relatively high pH optima (pH = 5-9) for activity and many have either an absolute requirement for, or are stimulated by Ca^{2+} ion. The classic hydrolysis of α-1,4-glycosidic linkages in pectin is catalyzed by a combination of pectinmethylesterase and endo-or exopolygalacturonases (*68,70,71*). The existence of polymethylgalacturonases which could hydrolyze polymethylgalacturonate is still under dispute (*68*). A small family of endopolygalacturonases that are highly active in hydrolysis of protopectin have been termed protopectinases (*72*). The pectinmethylesterases (*69*) are carboxylic acid esterases. The products of their action are de-esterified pectin containing increased amounts of carboxylic acid groups, methanol and protons from ionization of carboxylic groups. These enzymes are produced by many microorganisms and plant tissues (*69*). The presence of sufficient amounts of pectinmethylesterase appears to be very important for efficient solubilization and saccharification of pectin rich tissues (*68,70*). The pectinmethylesterase prepares the demethoxylated substrate for polygalacturonases and polygalacturonate lyases (*70*). These enzymes in turn remove end product inhibition of pectinmethylesterase by hydrolyzing demethoxylated pectin to D-galacturonic acid or very short oligogalacturonides which are no longer inhibitory (*68-70*). Very strong synergism between pectinmethylesterase and polygalacturonase or polygalacturonate lyase has been observed (*69,70*) both for pectin and apple cell walls as substrates. The enzymatic hydrolysis of apple cell walls with simple mixtures of purified pectinolytic enzymes also solubilizes considerable amounts of polymers enriched in arabinose, galactose, xylose and rhamnose (*60-63*) and disintegrates the tissue.

The next issue in structure and hydrolysis of fruit and tuber processing residues is the structure and attachment of hemicelluloses rich in arabinose, galactose and perhaps xylose in middle lamella and primary and secondary cell walls of these tissues. Side chains rich in arabinose and galactose are thought to be present in "hairy" regions of pectin, but hemicelluloses rich in these neutral sugars appear to exist as well. Isolation of arabinose from sugar beet and galactan from citrus peel has been mentioned already. The structure and composition of these polymers is relatively unclear at the present time, mainly due to difficulties with extraction and purification (*57,58*). Lack of well defined substrates also hinders isolation and characterization of appropriate hydrolytic enzymes (*73,74*). The observation pertinent to the structure and enzymatic depolymerization of cell walls in processing residues discussed here is the lack of pectin release when all three tissues are treated with purified endo-galactanase and endo-arabinanase enzymes (*61-63,75*). These results indicate that a large part of polymers containing arabinose and galactose may be covalently attached to pectin or enmeshed in it, but the same part of these polymers does not appear to crosslink the pectin to other cell wall components by covalent bonds. The selective enzymatic hydrolysis of cell walls of apple and citrus fruit which could release pectin would provide an important technical alternative to partial hydrolysis of these tissues with dilute mineral acids which is commercially used for the extraction of this valuable polysaccharide. The importance of xylanase activities in enzymatic hydrolysis of these tissues is unclear at the present time due to uncertain

structure of xylose containing polymers and xylanolytic activities of some purified cellulase enzymes (*62,63,76*).

Cellulose is the second most abundant polymer in cell walls of apple, orange and sugar beet tissues (Table II) but its structure in these tissues has hardly been investigated (*77*). The enzymatic hydrolysis of cellulose fibers has been investigated rather extensively during the last 25 years and the results have been summarized in recent reviews (*7,8,76,78,79*). The depolymerization of cellulose fibers requires cooperative hydrolysis by endo- and exo-ß-1,4 glucanase enzymes. Due to insoluble and partially crystalline nature of this substrate, the reaction is confined to the surface and proceeds rather slowly. These problems are compounded by encasing of cellulose fibers in the matrix of hemicelluloses and pectin which have to be depolymerized and dissolved before cellulase enzymes can access and depolymerize cellulose. Therefore investigations pertinent to our discussion (*48,50,60-63,70,80-88*) indicate that there is a significant synergism between cellulolytic and pectinolytic enzymes during hydrolysis of cell walls in citrus, apple and sugar beet tissues. The synergism of cellulolytic and pectinolytic enzymes has been reported for all three tissues and both purified and crude enzyme preparations. The crude pectinase preparations appear to be more effective in maceration and solubilization of these tissues than commercial cellulase preparations, and at least one commercial pectinase enzyme (*50,89,90*) contains sufficient amounts of cellulolytic and other hydrolytic activities that it can hydrolyze and solubilize fruit tissues without added cellulase. The addition of ß-glucosidase (*45,81*) can decrease the strong end-product inhibition of currently available cellulases, allow efficient hydrolysis at higher concentrations of substrate solids, and therefore allow production of concentrated sugar solutions. There are many other hydrolytic activities, notably esterases, glycosidases, and debranching enzymes, which are necessary for complete hydrolysis of pectin-rich plant tissues. These enzymes are not covered in this review.

It may appear that the sheer number of different enzymes necessary for complete hydrolysis of pectin-rich plant cell walls would prevent commercial development of enzymatic mixtures for maceration and solubilization of these tissues. Fortunately the key polymers (cellulose, hemicellulose and pectin) have been available to microorganisms mainly in the form of plant tissues, so microorganisms that decompose cellulosic materials (both pectin- and lignin-rich) have to secrete a complex mixture of enzymes to derive significant energy from hydrolysis and assimilation of these tissues. The crude exocellular enzyme preparations used in industry therefore usually contain numerous additional hydrolytic activities besides the ones used for their marketing (*50,70,89-91*). The presence of other hydrolytic activities makes commercial pectinase and cellulase enzymes very valuable for applications described here, because the development of efficient hydrolytic mixtures from individual enzymes would be a complicated and expensive task.

There are two other approaches that have been or can be used to augment the activity of exogenous enzymes in hydrolysis of pectin rich tissues reviewed here. One approach involves exploitation of endogenous hydrolytic enzymes produced in fruits and tubers. The second one involves chemical pretreatments which can solubilize, modify or depolymerize polysaccharides in cell walls and turn them into more active or simpler substrates.

Endogenous Hydrolytic Enzymes. The pectinmethylesterases appear to be ubiquitous in tissues of pectin rich fruits and tubers (*69,70*) where they are probably involved in growth, enlargement and eventual senescence. Acetylesterase active on acetylated pectin is commercially produced from orange peel, as is pectinmethylesterase (*92*). Endo- and exo-polygalacturonases present in many other fruit tissues appear to be absent from orange peel. Other hydrolytic enzymes may be present as well, but either have not been assayed or escaped detection (*93*).

Apple fruit provides a greater variety of endogenous hydrolytic enzymes (*94*). Besides pectinmethylesterase, exo-polygalacturonase (*70*), ß-1,4 glucanase (*94*) and numerous glycosidases (*95*) have been detected in apple fruit. Some of these may be active in hydrolysis of cell wall polymers, because histochemical investigations (*80*) indicate that middle lamella is depolymerized and dissolved during ripening of apples. The endogenous hydrolytic enzymes will be of lesser importance in enzymatic hydrolysis of leached beet cosettes, because they are probably inactivated by hot water during the leaching step.

Interaction of endogenous and exogenous hydrolytic enzymes can provide synergistic effects in hydrolysis of pectin rich fruit and tuber tissues, provided that pH and temperature optima of these enzymes are properly matched.

Another avenue for increased hydrolysis and solubilization of pectin-rich plant cell walls by enzymes is provided by chemical pretreatments.

Chemical Pretreatments. Many polysaccharides in pectin-rich plant cell walls are susceptible to acid-catalyzed hydrolysis. The extraction of pectin from apple and citrus processing residues by hot, dilute mineral acids is the basis of commercial pectin production (*96*). Treatment with hot acid also breaks the crosslinks in pectin-rich cell walls and both depolymerizes and solubilizes hemicelluloses containing arabinose, galactose, xylose and glucose. Only cellulose fibers are quite resistant to the action of dilute mineral acids. The treatment with dilute sulfuric acid has been used for partial solubilization of orange peel (*83,87,97*) either in applications where solubilized carbohydrates have been used for single cell protein production (*97*) or as a pretreatment to obtain enhanced enzymatic saccharification of carbohydrates (*83,87, 97*). Hydrolysis of citrus peel catalyzed by carbon dioxide or indigenous organic acids at elevated temperatures and pressures has been patented (*98*). Similar treatments with dilute mineral acids have been tested for solubilization and pretreatment of apple pomace (*47,56,99,100*) and sugar beet pulp (*46,101,102*). The pretreatment with dilute sodium hydroxide has also been tested for all three residues (*45,83,85,102,103*). The treatment with sodium hydroxide de-esterifies pectin. It also extracts pectin and hemicelluloses by the base catalyzed cleavage of glycosidic bonds between galacturonic acid units and solubilizing action on hemicelluloses.

The chemical pretreatments of pectin rich tissues will have to be carefully evaluated in the future because with newer enzyme preparations they are not necessary, the results are highly influenced by the composition of enzyme preparations and chemical pretreatments add to the complexity and cost of the saccharification. It is probable, however, that chemical pretreatments can increase rates of hydrolysis and decrease consumption of hydrolytic enzymes which are both important economic considerations.

On the technical side, the enzymatic hydrolysis of pectin rich processing residues appears to be much easier than the hydrolysis of lignified cellulosic substrates. Pectin and hemicelluloses provide some barrier to cellulolytic enzymes, but these polymers are easily hydrolysed and solubilized by pectinolytic and hemicellulolytic enzymes. Severe chemomechanical pretreatments that must be used before polysaccharides in lignified plant tissues become susceptible to hydrolysis by enzymes do not appear to be necessary with pectin rich tissues. Inhibition of enzymatic hydrolysis of apple tissues by soluble phenolic compounds has been observed, but this inhibition can be removed by aeration and holding of the pulp which leads to polymerization and precipitation of these compounds (*25,70*).

The low content of insoluble cellulose and lignin leads to rapid liquefaction of these tissues during enzymatic hydrolysis even at high (>8%) concentrations of solids. Our work with orange peel (*50,81*) also indicates that very low amounts of enzymes are needed for relatively rapid (<12 hrs) saccharification of the peel. There is not enough data with thoroughly characterized enzyme preparations to compare rates of enzymatic hydrolysis of cellulose in lignified and pectin rich plant tissues at the present time, but recent industrial and academic interest should remedy the situation in the near future. Treatments with pectinolytic enzymes are used in the fruit juice industry (*25,28,70*) and the GRAS (i.e. generally recognized as safe) status of many of these enzymes can only lead to the expansion of their utilization.

Another important difference between the enzymatic hydrolysis of lignocellulosic tissues and sugar rich residues discussed here is a small contribution of glucose from hydrolysis of cellulose to the overall yield of soluble sugars. Inspection of Tables I and II shows that cellulose is not the most important contributor to soluble sugar production as is the case with lignocellulosic substrates. The release of soluble sugars retained in tissues of citrus and apple processing residues is of utmost importance, followed by the hydrolysis of pectin and hemicelluloses. Complete hydrolysis of cellulose adds only a small percentage to the overall sugar yield and may not be as important as it is with lignocellulosic substrates. Similar considerations apply to enzymatic saccharification of unleached sugar beets, where soluble sugar (sucrose) is by far the most abundant component of total dry solids and cellulose amounts to only a few percent. Therefore, the emphasis in enzymatic hydrolysis of tissues discussed here is actually on maceration and disintegration of tissues and cell walls by pectinolytic and hemicellulolytic enzymes to allow release of entrained soluble sugars. Hydrolysis of cellulosic fibers is of lesser importance and possibly may be omitted. The enzymatic hydrolysis of pectin is more efficient than similar hydrolysis of cellulose and requires very small amounts of enzyme (*104*), which is a very important economic consideration. The formation of galacturonic acid and much smaller amounts of acetic acid by the action of esterases lead to a significant increase in hydrogen ion concentration during enzymatic hydrolysis. The pH of the reaction mixtures decreases to the 3.2-3.5 range, unless significant amounts of base are added. Therefore the preferable enzymes for efficient hydrolysis of pectin rich tissues should be acidophilic and retain activity at pH of 3-4. However, the release of galacturonic acid and five-carbon sugars from hemicelluloses complicates subsequent utilization of enzymatic hydrolysates from juice processing residues. The hydrolyzates may be utilized as feed supplements for ruminants and chicken (105), but there may be problems with other farm animals such as pigs (*105*) which cannot digest five carbon

sugars and galacturonic acid. Microorganisms are adapted to utilization of a broad spectrum of sugars, consequently microbial conversions to fermentation products offer a greater variety of options. Although individual microorganisms may have difficulties with utilization of some sugars as is discussed below or display a sequential (diauxic) pattern of sugar utilization they offer many options for the conversion of mixed sugars to value added products. The conversion options that have received significant attention are summarized below.

Microbial Conversions

Background on the fermentability of enzymatic hydrolyzates is provided by extensive research and industrial experience with fermentations of expressed or leached juice, which in the case of apples and sugar beets has been used for industrial production of ethanol. Fermented (hard) apple cider is a common product in apple producing countries (*25*) and productivity of sugar beets is so high that they have been considered from time to time a resource for the production of the fuel ethanol (*106-109*). No serious problems have been encountered with these fermentations and the high content of mineral nutrients in apple and beet juice often makes addition of nutritional supplements unnecessary.

The fermentation of citrus peel and peel juice has been hindered by the presence of antimicrobial compounds, mainly limonene, in peel oil (*110-117*). Levels of limonene as low as 50-100 ppm are strongly inhibitory to yeast (*110*) and anaerobic bacteria (*112-117*). The limonene in peel juice is not inhibitory to aerobic cultures because it is stripped by aeration. It can also be removed by steam stripping which occurs during the concentration of peel juice to molasses in multiple effect evaporators (*23*). A portion of peel molasses is used for industrial ethanol production in Florida and Brazil (*23,111*). We also observed (*81*) that limonene can be removed from peel hydrolyzates by simple filtration.

Besides fermentations of apple juice, several studies dealt with fermentation of apple pomace to ethanol (*38,118,119*) with (*119*) or without (*38,118*) enzymatic hydrolysis. Production of citric acid (*39,120*), biogas (*35*) and single cell protein (*121*) from apple pomace and apple distillery slops (*121*) has also been investigated. Similar studies of ethanol (*46,107,122,123*), biogas (*124*) and single cell protein (*101,125,126*) production from sugar beet pulp have been conducted. Conversion of citrus processing wastes also received considerable attention. Highest interest has been in single cell (fungal) protein production (*127-140*) including cultivation of mushrooms (*141-145*), while anaerobic digestion (*113,115,146,147*) and ethanol production (*81,148*) have been investigated much less. The coupling of enzymatic hydrolysis with ethanolic fermentation by yeasts has not been a very productive choice for all three residues. Yeasts ferment efficiently only six carbon neutral sugars (*149,150*). They generally do not ferment galacturonic acid and five carbon sugars, such as arabinose, and xylose (*151*), although a few strains which ferment concentrated xylose solutions under microaerophilic conditions have been identified (*152*). The increase in the yield of six carbon neutral sugars (glucose and galactose) fermentable by yeasts is only modest after enzymatic hydrolysis of citrus and apple residues (see Tables I and II) or unleached sugar beet pulp. This small increase in the yield of fermentable sugars accounted for the modest (0-30%) increase in observed

ethanol yields when enzymatic hydrolysis and yeast fermentation were combined (*46,81,119,122,148*). The situation can improve with application of genetically engineered bacteria which have been constructed for fermentation of five carbon sugars to ethanol (*153*) or with genetic development of yeasts for fermentation of these sugars. Homofermentative production of lactic acid as a value added product suffers from similar limitations in the pattern of sugar utilization as ethanolic fermentation (*154*), even though xylose can be fermented to a mixture of lactic and acetic acid with smaller amounts of ethanol (*155*). Acetone-butanol fermentations can utilize all sugars in enzymatic hydrolysates discussed here, but a large increase in acetate formation has been observed during acetone-butanol fermentation of galacturonic acid (*156,157*). The development of fermentations for other products may require testing on a strain basis (*156*).

Increased attention needs to be paid to isolation and development of microorganisms utilizing arabinose and galacturonic acid. Arabinose is present in hydrolysates of both xylan- and pectin-rich plant tissues, and galacturonic acid is obviously a major sugar in hydrolysates of pectin rich tissues, some of which have been discussed here.

Conclusions

Pectin rich residues from the production of fruit juices and beet sugar offer a unique opportunity for the production of sugars by enzymatic hydrolysis of cellulosic substrates. The absence of lignin and low content of cellulose make these residues highly susceptible to depolymerization and solubilization by mixtures of pectinolytic and cellulolytic enzymes. These enzymes usually contain additional hemicellulolytic activities needed for depolymerization of arabinose, galactose and xylose rich polysaccharides which are also present in cell walls of these residues. The processing residues from apple and citrus juice production contain significant amounts of soluble sugars which are also effectively released by enzymatic treatment. High yields and relatively high rates of enzymatic depolymerization of carbohydrates in these tissues have been observed without any chemical pretreatment. High specific activity and low end product inhibition of commercial pectinolytic enzymes allow production of relatively concentrated sugar solutions at low enzyme loadings. These enzymatic hydrolysates appear to be suitable substrates for further microbial conversion to value added products. The main challenge in the development of microbial conversions of these hydrolysates is the identification and development of microorganisms utilizing galacturonic acid and arabinose which are abundant components of these hydrolysates and quite abundant in many other plant tissues.

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